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NEX77CIP2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	GOLD ET AL.		
SERIAL NO.:	09/616,284	EXAMINER: FORMAN, B.J. Ph.D.	
FILED:	JULY 14, 2000	ART UNIT: 1655	
FOR:	METHOD AND APPARATUS FOR THE AUTOMATED GENERATION OF NUCLEIC ACID LIGANDS	CONF. NO.: 6509	

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT AND RESPONSE

Dear Sir:

In regard to the referenced application and the office action of August 15, 2001, Applicants respectfully request entry of the following amendments.

AMENDMENTS

Please cancel claims 1-17 without prejudice as to the subject matter contained therein.

18. (Amended) A method for identifying a nucleic acid ligand that photocrosslinks to a protein from a candidate mixture of nucleic acids, wherein each member of said candidate mixture contains a photoreactive group, said method comprising:

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37 CFR 1.8
CERTIFICATE OF MAILING
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a) contacting said candidate mixture with said protein, wherein nucleic acids having an increased affinity to the protein relative to the candidate mixture form nucleic acid-protein complexes with the protein;

b) irradiating said complexes, wherein said nucleic acid-protein complexes crosslink;

c) partitioning the photocrosslinked nucleic acid-protein complexes from the remainder of said candidate mixture; and

d) identifying a nucleic acid ligand that photocrosslinked to the protein;

wherein steps (a)-(c) are performed at one or more work stations on a work surface by a cartesian robotic manipulator controlled by a computer.

REMARKS

By the foregoing amendments, claim 18 has been amended to correct typographical errors. Claims 18 and 19 are pending in the case.

The Restriction Requirement

In response to the restriction requirement, the Applicants confirm the election of group III, claims 18 and 19, without traverse. By the foregoing amendments, non-elected claims 1-17 have been canceled.

Claim Objections

Claim 18 has been objected to as containing two inappropriate periods. By the foregoing amendments, these informalities have been corrected.

The Rejections under 35 USC § 103

Claims 18 and 19 stand rejected under Section 103(a) as being unpatentable over Cox et al. (1998) Biotechnology Prog. 14:845-50 in view of Hanna (1989) Methods in Enzymology 180:383-405. The Examiner alleges that Cox et al. teach a SELEX-like method, wherein method steps are performed at work stations by a Cartesian robotic manipulator, and that Hanna teaches photocrosslinking of a nucleic acid to a protein so as to identify the nucleic acid. Applicants respectfully traverse this rejection.

Cox et al. describe automation of a method for selection of nucleic acids that are complementary to a target oligonucleotide. Thus, Cox et al. do not describe a SELEX-like or aptamer selection process. While the introduction of the Cox et al. publication refers generally to selection of nucleic acid aptamers, as opposed to nucleic acid hybridization partners, the authors do not attempt to automate an aptamer selection method. Instead, they state that "molecules such as proteins will undoubtedly prove to be more difficult selection targets" than oligonucleotide hybridization partners (page 848, last full paragraph). Thus, at most, Cox et al. is an invitation to experiment with automation of a selection method to obtain nucleic acid aptamers to protein. Given that the processes of nucleic acid hybridization and nucleic acid aptamer-protein binding are completely different in their chemical nature and kinetics, and given that partitioning of double-stranded oligonucleotides from unhybridized, single-stranded oligonucleotides, is essentially different from partitioning of aptamer-protein complexes from free nucleic acids, it is submitted that Cox et al. is far too speculative to reasonably predict the success of automation of a SELEX-like method.

Further, Cox et al. make no mention whatsoever of the added step of irradiating oligonucleotides containing photoreactive groups so as to form crosslinked aptamer-target complexes, as is presently recited in claims 18 and 19. There is no apparatus in Cox et al. for irradiating an array of mixtures of nucleic acids and target proteins. The use of a candidate pool of oligonucleotides with photoreactive groups, the mixing and complexing of such oligonucleotides with protein targets to form complexes, and the irradiation of such complexes, introduce yet more steps and variables into the automation process. These additional steps introduce more uncertainty in predicting whether a SELEX-like method with a photocrosslinking step could be successfully automated.

Hanna does nothing to cure the deficiencies of Cox et al. Hanna describes photoaffinity cross-linking methods to characterize interactions between the RNA and protein of naturally-occurring complexes such as ribonucleoprotein complexes. Hanna's experiments are designed to confirm a suspected naturally-occurring RNA-protein interaction, or to define through footprinting which nucleotides interact with which amino acids in the complex. Hanna is not concerned with the screening of millions of synthetic

oligonucleotides to identify those nucleic acid ligands with the highest affinity to protein targets. At page 389, third full paragraph, Hanna states:

> By placing the photoreactive cross-linking group on the RNA molecule, it is possible to study RNA-protein interactions with proteins in their native conformations. Care must be taken, however, to maintain the **native** conformation of the RNA during introduction of the cross-linker.

The emphasis of Hanna on naturally-occurring RNAs and footprinting of such RNAs and their ligand proteins is also evident in this passage from page 393, first paragraph:

> [T]he introduction of photoreactive nucleotides into a RNA chain can be used for (1) identification of protein(s) . . . that interact with or bind to a specific RNA, (2) identification of protein domain(s) or amino acid(s) involved in a specific RNA interaction, and (3) identification of RNA regions or nucleotides involved in a specific protein interaction.

Hanna makes no reference to a SELEX-like process for selection of aptamer or nucleic acid ligands to a target (e.g., protein or non-oligonucleotide), or how crosslinking might be useful in such a process. Further, Hanna makes no suggestion regarding automation of her crosslinking or identification methods. She describes no apparatus for irradiation of an array of tubes containing nucleic acids and targets.

For the foregoing reasons, Applicants respectfully submit that there is insufficient suggestion in the Hanna and Cox et al. references to support a case of *prima facie* obviousness. At most, these two references might make the claimed invention obvious to try. Hanna and Cox et al. provide only an invitation to experiment, by providing only very general guidance about exploring a new technology or a general approach that may be promising. It is well established that obvious to try is not the appropriate standard in an obviousness determination (*In re O'Farrell*, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988)).

Further, there is no suggestion in the references that their teachings be combined. There is no teaching, express or implied, in Hanna that her photocrosslinking methods could be useful in screening millions of nucleic acids, or that such screening method could be automated. There is no express or implied teaching in Cox et al. that a photocrosslinking step could be incorporated into an automated nucleic acid selection

process. Without such a suggestion, *prima facie* obviousness has not been established (*Gambro Lundia AB v. Baxter Healthcare Corp.*, 42 USPQ2d 1378 (Fed Cir. 1997)).

The Examiner asserts that motivation to combine the references arises from Hanna's teaching that crosslinking permits one to "obtain weak or transient nucleic acid-protein complexes in their native conformation and obtain weak or transient nucleic acid-protein interactions . . . for the expected benefit of obtaining otherwise unobtainable complexes" (page 5, final sentence, of office action). Applicants respectfully point out that the purpose of SELEX and photoSELEX is to obtain aptamers with greater affinity for target proteins, not nucleic acids that weakly or non-specifically bind to the target proteins. Thus, it is respectfully submitted that Hanna does not provide the necessary motivation to combine the references to obtain the claimed invention. Without a motivation to combine the references in such a manner so as to arrive at the claimed invention, *prima facie* obviousness cannot be established (*Ex parte Hiyamizu*, 10 USPQ2d 1393, 1394 (PTO Bd. App. Intf. 1988)).

Applicants submit that even if a case of *prima facie* obviousness had been established, it is rebutted by evidence of unexpected results (*In re Dillon*, 16 USPQ2d 1897 (Fed. Cir. 1990)). The unexpected result is associated with the automation of the partitioning step of photoSELEX. Manual photoSELEX had been carried out using gel purification for the partitioning step to separate crosslinked nucleic acid-protein complexes from free nucleic acids (see col. 17, line 57 to col. 18, line 19 of U.S. Pat. 6,001,577, submitted herewith in the Information Disclosure Statement). Gel electrophoresis is not generally amenable to automation because of the difficulties associated with the loading of gels and the excision of bands. Moreover, because these processes are time-consuming, it would likely be the rate limiting step in any automated SELEX process that employs it. The inventors of the subject invention overcame this obstacle in two ways: solution photoSELEX and bead photoSELEX. Both are systems in which the partitioning step is fluid based, permitting its automation.

In bead photoSELEX, the partitioning step involves the fixing of target to paramagnetic beads, and the separation of free nucleic acid from crosslinked nucleic acid-protein-bead complexes using the application of a magnetic field. In solution photoSELEX, the crosslinking is carried out with nucleic acid and target in solution, and

then the protein is captured with a paramagnetic bead with appropriate ligand to the target.

In the subject application, there is no exemplification of the automated photoSELEX method. However, USSN 09/993,294, filed November 21, 2001, by the same inventors as the subject application, demonstrates that the enabling statements set forth in the subject application relating to automation of photoSELEX are in fact borne out by experimental results. Enclosed herewith is a copy of USSN 09/993,294, and the Examiner's attention is directed to Examples 13-15, wherein automated photoSELEX is successfully practiced on a number of protein targets. Applicants also wish to point out that it is permissible to submit experimental data (e.g., USSN 09/993,294) collected by inventors after the filing date of an application, to support enabling statements made in an application (*In re Marzocchi*, 169 USPQ 367, note 4 (CCPA 1971)).

In view of these experimental results demonstrating that photoSELEX can unexpectedly be automated via the development of alternate fluid-based strategies for carrying out the partitioning step, it is submitted that the claimed invention is non-obvious.

Claims 18 and 19 also stand rejected under Section 103(a) as being unpatentable over Gold et al., U.S. Pat. 5,475,096, in view of Cathcart et al., U.S. Pat. 5,443,791, and Hanna (1989), supra. The Examiner alleges that Gold et al. teach the SELEX method; that Cathcart et al. teach an automated method for partitioning and identification of a nucleic acid binding (hybridization) partner; and Hanna teaches modifying a nucleic acid group to permit photocrosslinking to a protein. Applicants respectfully traverse this rejection.

Gold et al. ('096) does teach the basic SELEX method for selection of nucleic acid ligands of higher affinity to targets (proteins or non-nucleic acids), from thousands or millions of candidate oligonucleotides, using reiterative rounds of complexing, partitioning, and amplification. Gold et al. does not teach automation of this process. Additionally, Gold et al. has no description relating to photocrosslinking of nucleic acid ligands to their targets.

Cathcart et al. teaches an automated method, but not automated SELEX. Rather, Cathcart et al. teaches use of robotic pipetting and electrophoresis apparatus to conduct a

liquid version of Southern blotting to detect genes that hybridize via Watson-Crick base pairing to a labeled probe (see Appendix A of Cathcart et al.). Cathcart et al. make no mention of aptamers or nucleic acid ligands. Thus, the combination of Gold et al. and Cathcart et al. is insufficient to suggest automation of a SELEX-like method for selection from a candidate pool, of those oligonucleotides having a higher affinity for the target (protein or non-nucleic acid).

Further, Cathcart et al. have no description relating to photocrosslinking of oligonucleotides to proteins or other non-nucleic acid targets, or for that matter, to hybridization partners.

Hanna does nothing to cure the deficiencies of Gold et al. and Cathcart et al. As discussed above, Hanna describes photoaffinity cross-linking methods to characterize interactions between the RNA and protein of naturally-occurring complexes such as ribonucleoprotein complexes. Hanna's experiments are designed to confirm a suspected naturally-occurring RNA-protein interaction, or to footprint the nucleotides and amino acids directly involved in the interaction. Hanna is not concerned with screening of millions of synthetic oligonucleotides to identify those nucleic acid ligands with the highest affinity to protein targets. Hanna makes no reference to a SELEX-like process for selection of aptamer or nucleic acid ligands to a protein or non-oligonucleotide target, or to the use of crosslinking in such a process. Further, Hanna makes no suggestion regarding automation of her cross-linking or identification methods.

It is therefore submitted that the combination of Gold et al., Cathcart et al. and Hanna are insufficient to establish *prima facie* obviousness because there is insufficient suggestion to combine their teachings to obtain the claim invention (*Gambro Lundia AB, supra*). As discussed above, the Examiner contends that the motivation to combine the references comes from Hanna. That alleged motivation is the goal of obtaining weak or transient nucleic acid-protein interactions for the expected benefit of obtaining otherwise unobtainable complexes. As pointed out above, both SELEX and photoSELEX are concerned with obtaining oligonucleotides that bind with increased affinity to their targets, rather than oligonucleotides that bind non-specifically or with low affinity to their targets. The skilled artisan interested in the SELEX method would not have been

motivated to adjust the method to obtain oligonucleotides having low or non-specific affinity.

Applicants submit that Gold et al., Cathcart et al. and Hanna at most provided an invitation to experiment in a new technology. Because these references neither discussed the desirability of automating photoSELEX in particular, nor pointed to specifically how that goal might be accomplished, it is submitted that these references at most made the claimed invention obvious to try, which, as discussed above, is not the correct standard under Section 103.

Finally, Applicants submit that even had *prima facie* obviousness been established, it is rebutted by the unexpected result of automation of the partitioning step of photoSELEX as discussed above. The subject inventors have developed two means of converting the gel electrophoresis partitioning step of manual photoSELEX to a fluid based system, thereby rendering it capable of automation. Evidence of the success of this method is submitted herewith in Examples 13-15 of USSN 09/993,294.

For the foregoing reasons, the Applicants respectfully request that both of the Section 103(a) rejections be withdrawn.

The Sequence Listing Requirement

Submitted herewith is a new Sequence Listing, a new computer readable form (CRF), and a new Statement under 37 CFR § 1.821, which are believed to remedy the error in the prior CRF.

Closing Remarks

Submitted herewith is a Petition for Extension of Time for 3 months with an authorization to charge Deposit Account No. 50-1643 for associated fees. Also submitted herewith is a Supplemental Information Disclosure Statement with an authorization to charge the Deposit Account. It is believed that no other fees are due with this submission. If this is in error, please charge Deposit Account No. 50-1643 for any necessary fees.

It is believed that the foregoing amendments and remarks bring the subject case into condition for allowance and notification of same is respectfully requested. If the

Examiner believes that it would expedite prosecution, she is invited to phone the undersigned at (303) 268-0066.

Respectfully submitted,

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Enclosures
cc: V. Appleby w/ encls.